STUDY ON THE EFFECT OF ULTRASOUND ON OXYGEN UPTAKE RATE (GAS-LIQUID MASS TRANSFER) OF SACCHAROMYCES CEREVISAE FERMENTATION

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ABSTRACT

The study on the effect of ultrasound on oxygen uptake rate (OUR) and gas-liquid mass transfer of *S.cerevisae* fermentation are reported objectives were focused on the producing ethanol from bioreactor by *S.cerevisae* fermentation. The main objective of this study was focused on the gas-liquid mass transfer coefficient (k_La) by a various type of solution, for example (water, glucose solution (50g/l) and fermentation broth). The relationship between k_La with an aeration rate and agitation speed were analyzed for ultrasound and without ultrasound application. Study showed that ultrasound at sonication regiment of 15 watts and 10% duty cycle were exposed to give a good relationship between k_La and fermentation. The profiles of OUR in the fermentation of *S.cerevisae* also reported. An application of ultrasound at power of 15 Watts gives a better improvement of OUR compared to without ultrasound. Ultrasound has a potential to assist an aeration rate and agitation speed of the fermentation.

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ABSTRAK

Kajian keatas kesan ultrabunyi pada kadar pengambilan oksigen (OUR), pemindahan jisim gas-cecair untuk fermentasi *S.cerevisae.* Objektif kajian ini telah difokuskan kepada penghasilan ethanol daripada fermentasi *S. cerevisae* dengan bioreaktor. Objektif utama kajian ini telah difokuskan kepada pemalar pemidahan jisim (k_La) untuk pelbagai jenis larutan, contohnya air, larutan glukosa (50 g/l) dan media fermentasi. Hubungan diantara k_La dengan kadar pengudaraan dan kelajuan pengadukan telah dianalisis dengan aplikasi ultrabunyi dan tanpa ultrabunyi. Kajian menunjukkan bahawa ultrabunyi pada rejimen sonikasi yang didedahkan pada 15 watt dan 1 minit/s memberikan hubungan yang baik diantara k_La dan fermentasi. Profil OUR dalam fermentasi *S.cerevisae* turut dilaporkan. Aplikasi ultrabunyi pada kuasa 15 watt memberikan penambahbaikan yang baik untuk OUR berbanding tanpa ultrabunyi. Ultrabunyi mempunyai potensi untuk membantu kadar pengudaraan dan kelajuan pengadukan dalam fermentasi.

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^{o}C	Celsius
ATP	Adenosine Triphosphate
BC	Before Christ
DIC	Differential Interference Contrast
DNS	Dinitrosalicyclic acid
DO	Dissolve Oxygen
FID	Flame Ionization Detector
HPLC	High Performance Liquid Chromatography
kHz	Kilohertz
MHz	Megahertz
OD	Optical Density
OUR	Oxygen Uptake Rate
STT	Straight Tip Connector
UV	Ultra Violet

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Sound of frequency >20 kHz is generally regarded of ultrasound and is audible to human. The upper limit of ultrasound frequency is not precisely defined but is commonly taken to be 5 MHz in gases and 500 kHz in liquids and solid (Mason,2002). The ultrasound maybe divided loudly into “low power” or “high power” ultrasound and “power” ultrasound. Ultrasound can influence the oxygen uptake rate (OUR) for the fermentation of yeast. (Y.Chisti, 2003). Ultrasound reported that can destroy microbial and others cells are the well-known effect has perhaps discourage research on possible beneficial effect of ultrasound on OUR system.

The research investigated the use of Ultrasound on OUR for *S.cerevisae* fermentation. An aeration rate and agitation speed effects on the fermentation could be studied and any observed effect by the ultrasound will be discussed. The aim is to identify the sonication regimen that might be suitable for enhancing the gas hold-up and attempt to elucidate the possible mechanisms involved in any productivity enhancement from *S.cerevisae* fermentation.

1.1.1 Yeast Cells

Yeast is a microscopic fungus, a single cell organism from natural plant which usually has a size of 5-10 micrometers in size (Charlie, 1998). Most of yeast cell has a simple morphology, whether in the form of an oval or rod form. *S.cerevisae* and *S. carlbergensis* are oval-shaped yeast cell, and *candida* yeast is the example of rod shaped (Parry and Pawsey, 1984) Charlie (1998) states that yeast species are different from each other, depending the shape and morphology of cell and how the yeast to metabolize different substrates and its reproduction.

Yeast can be found in grain of wheat, wheat products, silage, straw, soil and water and others. Yeast is facultative anaerobic, it can live and grown with or without oxygen. Yeast propagation is the process aerobic which is yeast will converts the sugar to oxygen and carbon dioxide and sufficient free energy useful for yeast cell growth through metabolic oxidation method. Increased in the cell volume or size is known as yeast cell growth. Growth of yeast culture was increase in the number of cell such as an overall increase biomass (Kratochvilova, 1990). In yeast, ethanol causes an increase in hydrogen in flux across the plasma membrane of cells

suspended in water. Since ethanol does not accumulate within yeast cells but rapidly diffuse across the cell membrane, direct inhibition of glycolytic enzymes by intracellular ethanol is unlikely during fermentation which produces 12% (vol/vol) ethanol or less (Dombek, 1987)

1.1.2 S.cerevisae

Saccharomyces cerevisae is type of yeast that can be found in various forms such as pseudomycelia or as single cell organism. It was produce by isolated process which is from the grape's skin. Then, by multilateral budding, the cell was produce. *S. cerevisae* produces from one to four ellipsoidal, smooth walled ascospores. It can distinguish from others yeast based on accretion features and traits of physiological which principally the capabilities to ferment individual sugars (Molero, 1998). *S.cerevisae* is a high performance yeast on ethanol production, high protein content in living cell, high resistance on stress environment as low pH and high temperature (38 ^{o}C) (Klomklieng, 2011).

By the late 18th century, two yeast strains used in brewing had been identified as Saccharomyces *cerevisae,* so-called top fermenting yeast, and *S.carlsbergensis* bottom fermenting yeast. *S.cerevisae* has been sold commercially by the Dutch for bread making since 1780, while around 1800, the Germans started producing *S.cerevisae* in the form of cream. In the United States, naturally occurring airborne yeasts were used almost exclusively until commercial yeast was marketed at the Centennial Exposition in 1876 in Philadelphia, where Charles L.

Fleischman exhibited the product and a process to use it, as well as serving the resultant baked bread.

S.cerevisae is used extensively in fermentation to convert sugar to ethanol for the production of beverages and biofuel. It also used universally for industrial ethanol production because of ability to produce high concentration of ethanol and high inherent ethanol tolerance. (Watanabe, 2007). *S.cerevisae* is capable of very rapidly rates of glycolysis and ethanol production under optimal conditions, producing over 50 mmol of ethanol per h per g of cell protein. However, this rate is maintained for only a brief period during batch fermentation and declines progressively as ethanol accumulates in the surrounding broth (Dombek, 1987).

The word "fermentation" came from the Latin work *fevere* meaning "to ferment." Fermentation is a process that has been introduced since the ancient times and this method has been applied that time. Fermentation is a process of chemical change caused by organisms or their products, usually producing effervescence and heat. It also is process where the energy will extract from the oxidation of organic compounds like carbohydrates by endogenous electron acceptor which is commonly an organic compound. Otherwise, the respiration is donated the electrons to an exogenous electron acceptor, likes oxygen by using electron transport chain. This process occurs in mammalian muscle during time of intense where supplying of oxygen becomes limited, it resulting in the existence of lactic acid. It is important in anaerobic conditions when there is no oxidation phosphorylation to maintain the production of ATP (adenosine triphosphate) by glycolysis. Other than that, fermentation is also use as a method of food processing production of organic acids, pH-development and microbial growth in fermenting cereals.

1.1.3 Ultrasound

Ultrasound is acoustic (sound) energy in the form of waves which having a frequency that higher than upper limit of the human hearing range. From research that has been done, human ear only can detect the highest frequency is approximately 20 thousand cycles per second (20,000 Hz). This is where the sonic range ends, and where the ultrasonic range begins. There are many uses of ultrasound in others field such as ultrasound is used in electronic, navigational, industrial, and security applications (Santos, 2009).

High energy or 'power ultrasound' in the 20-100 kHz frequency range is used in many sonochemical processes. At high power input, ultrasound can rupture cells and ultra-sonication is a well-establish laboratory technique of cell disruption. High power of ultrasound can induced cavitations, generation of free radical and other mechanical and chemical effects. During cavitation's, micro bubbles form a various nucleation sites in the fluid and grow during the rarefaction phase of the sound wave. Then, in the compression phase, the bubbles implode and collapsing bubbles releases a violent shock wave that propagates through the medium (Chisti, 2003).

Using of low-power of ultrasound can enhance the production of ethanol by fermentation process. Ultrasonic power can enhanced ethanol production rate by reducing fermentation time compare to the use of the control bioreactor. Ethanol production increases in proportion to the increases of ultrasonic power under ultrasonic power supply at 20-30 kHz. Optimum power of ultrasound promotes membrane permeation efficiency on cells and it has been used to induce transfer of genetic material into live animal and plant cell. (Klomklieng, 2001)

The productivity of a biological process can be increases by using of ultrasound in specifically designed bioreactor. However, there are few studies on the effect of ultrasonic to performance of live microbial, especially under fermentation condition. Ultrasound is used in industry to analyze the uniformity and purity of liquids and solids. It can also be used for cleaning purposes. Subminiature ultrasonic cleaning instruments are used by some dentists during routine examinations. Ultrasound has a potential in many food-processing applications.

1.2 Problem Statement

In the fermentation of *S.cerevisae* for production of bioethanol. There are two process involve which are aerobic and anaerobic process. In anaerobic process, it can produce more ethanol and less biomass production. In this study, the research is focused to investigate the mass transfer coefficient (k_La) of the liquid. Beside that in aerobic process, it can produce more biomass (cell) and less ethanol production and it also depend on air and gas. Air and gas can effect aeration rate and agitation speed.

When aeration rate increase, the gas hold-up from the liquid can effects the rate of k_La Using of ultrasound can give better aeration rate and agitation speed, then it can improve the kinetic parameters of *S.cerevisae* fermentation.

Ultrasound can damage live cell, however, under suitable condition, sonication has been used to positively influence biochemical process particularly the live cell. Uses of the ultrasound in fermentation are relatively new and offer opportunity for enhancing the rate and productivity of ethanol. Because demand in ethanol is increasing year by years, this research will gives an overview of the new

technologies required and the advances achieved in recent years to bring ethanol towards industrial production to produce large amount of ethanol.

1.3 Research Objective

The aim for this study to investigate on the effect of ultrasound on oxygen uptake rate (gas liquid mass transfer) of *S.cerevisae* fermentation and study on the producing of ethanol from fermentation of *S.cerevisae.*

The objectives that must be achieved in this research are:

i. Determine the kinetic parameter of the *S.cerevisae* fermentation with/without ultrasound.

ii. To compare the kinetics parameter of *S.cerevisae* fermentation.

iii. Determine the relationship between ultrasound, aeration rate and agitation speed for *S.cerevisae* fermentation.

1.4 Scope Of Study

To achieve the objectives, few scopes have been identified in this research:

i. To investigate the effect of ultrasound on oxygen uptake rate (OUR) using various aeration rate and agitation speed in fermentation of *S.cerevisae.*

ii. To determine the kinetics parameter of the fermentation with/without ultrasound.

iii. To relate the rheological effect of fermentation with sonication regimens used for the fermentation.

1.5 Significant Of Study

The significant of study in the fermentation of *S.cerevisae* by using ultrasound. Fermentation is one of the techniques to produce ethanol, it is useful in our daily life such as in food processing and beverage, our transport need ethanol because it can produce fuel. Fuel ethanol production is expected to rise strongly and it will go along with an ever wider geographical spread. Ethanol is biodegradable without harmful effects on the environment, so this study did not give any side effect.

This study also will help government in developing countries because of the demand for ethanol production in the world. The purpose work identifies methods for implements ultrasound for enhancing the productivity by varying the aeration rate to agitation speed of the fermentation. An improve understanding is gained of possible mechanism of ultrasound induced enhancements due to gas-hold up in the fermentation improvement of the system.

CHAPTER 2

LITERATURE REVIEW

2.1 *S.cerevisae* (Yeast)

2.1.1 History of *S.cerevisae*

The word 'yeast' is used in many languages to describe roughly related to the phenomenon of fermentations. The word 'yeast' in the English language and the word 'Gist' in Netherlands is believed to come from the word 'zestos' in Greek. Which means is boiling, foaming, which is a common phenomenon during the fermentation process due to the release of carbon dioxide. (Quinn, 2005)

S.cerevisae has been introduced as an experimental organism in the mid-thirties of the 20th century and has since received increasing attention (Roman, 1981).

The elegance of *S.cerevisae* genetics and the ease of manipulation of yeast, and finally the technical breakthrough of yeast transformation to be used in reverse genetics, have substantially contributed to the enormous growth in *S.cerevisae* molecular biology (Siggersd,2008). *S.cerevisae* is a genus in the kingdom of fungi that includes many species of yeast.

The yeast has been used in the rocks, tombstones, stone and wooden toys from Ancient Egypt. Products such as beer and bread have begun served as food for the royal family among ancient Egypt since 6000 years ago (Davenport, 1980). The purpose of the use of yeast is for the brewing industry. Uses of yeast as a flavoring agent in soups repair has been used in Babylonia (Kocková-Kratochvílová, 1990). Evidence existence of microorganisms has been attributed to a grinding lens called Dutchman Antony Leeuwenhoek (1632-1723).

2.1.2 Genus/ Species of *S.cerevisae* (yeast)

In the yeast family, it can be classified in many genus/species. The genus include *Saccharomyces bayanus*, used in making wine, and *Saccharomyces boulardii*, used in medicine. The presence of yeast in beer was first suggested in 1680, although the genus was not named *Saccharomyces* until 1837. It was not until 1876 that Louis Pasteur demonstrated the involvement of living organisms in fermentation and in 1883, Hansen isolated brewing yeast and propagated leading to the importance of yeast in brewing. (Sofie M. G. Saerens,2010).

The genus of *S. cerevisae,* also can be found to over the years. Sugar mold or fungus is meaning of *Saccharomyces* and while *cerevisae* has its origin in the Gaelic